REMARKS

Claims 56-59, 61-63, 65-67, 69, and 92-94 were pending in the application. Claim 56 has been amended herein and claim 66 has been cancelled, without prejudice. Claims 1-55, 60, 64, 68, 70-91 have been cancelled, without prejudice, by previous amendment. Accordingly, after the amendments presented herein have been entered, claims 56-59, 61-63, 65, 67, 69, and 92-94 will remain pending. Support for the amendments to the claim can be found throughout the specification and in the claims as originally filed.

No new matter has been added. Any amendment or cancellation of the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Priority

Applicants acknowledge that the present invention claim priority to U.S. Serial No. 09/227,595, filed January 8, 1999 (Atty. Docket No. RPN-001) and U.S. Serial No. 08/595,590, filed on February 2, 1996.

Objection to the Oath/Declaration

The Office Action indicates that the oath/declaration is defective. In particular, a new oath/declaration in compliance with 37 C.F.R. 1.67(a) that identifies the application by application number and filing date is required. The Office Action further references the non-initialed and non-dated alteration that have been made to the oath.

Applicants are in the process obtaining a new oath/declaration in compliance with 37 C.F.R. 1.67(a). Applicants will submit the new oath/declaration as soon as it is available.

Rejection of Claims 56-59, 62, and 65-67 Under 35 U.S.C. §112, First Paragraph

Claims 56-59, 62, and 65-67 have been rejected under 35 U.S.C. §112, first paragraph because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims. In particular, the Office Action states that the specification "does not reasonably provide

enablement for a modified CTLA4-immunoglobulin fusion protein with just any substitution, addition or deletion of at least one residue in the immunoglobulin constant region."

Applicants respectfully traverse the foregoing rejection on the grounds that, based on the teachings in Applicants' specification, one of ordinary skill in the art would be able to use the claimed invention using only routine experimentation. The fusion proteins of the invention have been modified to reduce their ability to activate complement and/or bind to an Fc receptor. As such, the instant specification teaches the regions of the constant region that are important in mediating Fc receptor binding or complement activation (see *e.g.*, page 11, line 18 to page 13, line 18). Applicants also describe several screening assays which can be used to test the effectiveness of modifications, including an assay for measuring Fc receptor binding using the human cell line U937 (see page 45); a lytic assay for the measurement of complement activation (pages 46 and 47 of the specification); and assays which can be used to demonstrate that any modifications made to the constant region do not interfere with the ability of a modified fusion protein to modulate costimulation, *e.g.*, the T cell proliferation assay taught on page 47.

Applicants also teach methods by which reductions in constant region effector function can be achieved, *e.g.*, by substitution, addition, or deletion of amino acids, as well as, exemplary means of performing such modifications. Importantly, the specification defines the term "immunoglobulin constant (IgC) region-mediated biological effector function", as including biological responses which require or involve, at least in part, the constant region of an immunoglobulin molecule (see page 10, lines 29-34 of the specification). Applicants also disclose specific effector functions, such as, complement activation, Fc receptor interactions, opsonization and phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), release of reactive oxygen intermediates and placental transfer. Applicants teach, however, that while such effector functions are desirable in many immune responses, they are undesirable in situations where an immune response is to be downmodulated. Because the CTLA4-immunoglobulin fusion proteins of the invention exhibit reduced IgC region-mediated biological effector functions, these proteins are efficient agents for downregulating immune responses (see page 10, lines 36-38 of the specification).

Thus, while Applicants respectfully traverse the foregoing rejection, in the interest of expediting prosecution, and in no way conceding to the validity of the rejection, instant claim 56 has been amended to specify two types of constant region-mediated biological effector functions, *e.g.*, a protein having a reduced ability to activate complement or a reduced ability to bind to a Fc receptor. Applicants acknowledge that while the replacement of a single amino acid can, in some instances, result in alerted biological activity of the protein, the instant claims recite a functional limitation of the claimed composition. Thus, the assertion that "even a single amino acid substitution or what appears to be an inconsequential modification will often dramatically effect the biological activity of the protein" is not applicable to the pending claims, because the compositions are narrowly drawn to a modified fusion protein that retains a specific activity, *e.g.*, a protein having a reduced ability to activate complement or a reduced ability to bind to a Fc receptor.

As discussed above, Applicants provide evidence of the ability to generate a functional CTLA4-Ig fusion protein which has mutations in the Ig constant region that eliminate Fc mediated function, and provide guidance as to how to generate such mutations and screen for mutants which possess the desired activity. Thus, the instant specification provides sufficient guidance to support the full breadth of the invention as claimed. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

Rejection of Claims 56-59, 65-67, 69, 92 and 94 Under 35 U.S.C. §103(a)

Claims 56-59, 65-67, 69, 92 and 94 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Linsley *et al.* (U.S. Patent 5,434,131) and further in view of Gillies *et al.* (*Hum. Antibody.* Hybridomas 1:47-54, 1990) and Freeman *et al.* (U.S. Patent No. 6,130,316) and Canfield *et al.* (*J. Exp. Med.* 173:1483-1491, 1991) and Lund *et al.* (*J. of Immunol.* 147:2657-2662, 1991) and Duncan *et al.* (*Nature* 332:738-740, 1988). This rejection is respectfully traversed.

Summary of §103 Rejections Based on the Cited Art

The Office Action states that the invention as a whole was "prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made, as evidenced by the references."

The Office Action relies on Linsley *et al.* as teaching "cloning and expression of human CTLA4, including the extracellular domain which is defined as amino acids 1-125." Linsley *et al.* also disclose the "formation and expression of nucleic acids encoding an immunoglobulin fusion protein comprising the extracellular domain of CTLA4 and the constant regions of human IgC 1, including the hinge region, CH1, CH2, and CH3 domains." The Examiner admits that Linsley *et al.* "does not teach substitutions in the Fc region at specific positions 234, 235, 237." According to the Office Action, "these deficiencies are made up for in the teachings of Gillies *et al.*, Freeman *et al.*, Canfield *et al.*, Lund *et al.*, and Duncan *et al.*"

The Office Action relies on Gillies *et al.* as teaching "modifications to chimeric antibodies, such as deletion of the CH2 domain or substitution of serine for cysteine residues in the hinge region to reduce constant region effector functions such as Fc receptor binding and complement fixation/activation which in turn reduces other effector functions such as ADCC and complement mediated lysis which Gillies *et al.* teach to be desirable for *in vivo* applications."

The Office Action relies on Freeman *et al.* as teaching "molecules of CTLA4Ig fusion proteins ... and B7-2 immunoglobulin fusion proteins and modifications to the CH2 domain ... at residues 234, 235, and 237 and at cysteines in the hinge to eliminate Fc binding. The Leu at position 234 was replaced by Ala, the Leu 235 was changed to Glu and Gly at position 237 was changed to Ala."

The Office Action relies on Canfield *et al.* as teaching the "Leu at position 234 and Leu at position 235 are critical as well as Pro at position 331." Canfield *et al.* also teaches "substitutions of several of these positions, including Glu 235 and Ser at 331, to reduce Fc

binding and discuss the role of these regions in the engineering of antibodies with desired binding properties."

The Office Action relies on Lund *et al.* as teaching "substitutions of Ala for Leu at 234, Ala for Leu at 235, and [A]la for Gly at 237 to reduce Fc binding functions."

The Office Action relies on Duncan *et al.* as teaching "substitutions of Ala for Glu at position 318, Ala for Lys at 320, Ala for Lys at 322 to reduce Fc binding function."

Summary of the Arguments Presented: The Pending Claims Are Unobvious Over the Cited Art

To establish a *prima facie* case of obviousness for the claimed invention, there must have been some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings in the manner proposed by the Office Action. Second, there must have been a reasonable expectation of success at the time the invention was made. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. See M.P.E.P. 2143. The prior art must suggest "to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process" and "[b]oth the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." *In re Dow Chemical Co.* 837 F.2d 469. 473, 5 U.S.P.Q.2d 1529, 1531 (Fed.Cir. 1988).

It is Applicants' position that the Office Action has failed to establish a *prima facie* case of obviousness, because at the time the invention was made, there was no motivation in the art to combine the teachings of the references in the manner proposed by the Office Action. In particular, the references cited in the Office Action do not teach or suggest the pending claimed compositions of a modified CTLA4-immunoglobulin fusion protein comprising a first peptide having at least one CTLA4 activity and a second peptide having a Cγ4 immunoglobulin constant region which is modified to reduce at least one constant region-mediated biological effector function, wherein said effector function is selected from the group consisting of: (a) a reduced

ability to activate complement and (b) a reduced ability to bind to a Fc receptor, relative to an unmodified CTLA4-immunoglobulin fusion protein. The claims are further drawn to a CTLA4-immunoglobulin fusion protein comprising a first peptide having at least one CTLA4 activity and a second peptide comprising an immunoglobulin constant region wherein the immunoglobulin constant region comprises the CH1 domain, hinge region, CH2 domain, and CH3 domain from a Cγ4 heavy chain. The modified CTLA4-Ig forms of the present invention represent an improvement over the prior art CTLA4-Ig fusion proteins, because the modifications to the constant region eliminate the detrimental side effects associated with complement activation and Fc receptor binding.

CTLA4-Ig fusion proteins in the prior art, which lack modifications that eliminate Fc mediated responses, were demonstrated effective and useful at inhibiting immune response both *in vitro* and *in vivo*. In addition to a lack of any established or suggested improvement in the properties of the CTLA4-Ig fusion protein by the prior art, the prior art lacks any established need for modification of the then existing CTLA4-Ig fusion proteins.

Furthermore, the teachings of the prior art do not indicate a reasonable expectation of success at making the claimed invention, due to the possibility that the modifications to the Fc region will disrupt the necessary structure of the CTLA4 portion of the fusion protein. The prior art teaches that modifications to the constant region of whole antibodies affect the structure of the entire molecule. For example, Gillies *et al.* teaches that mutations in an antibody which eliminate effector mediated interactions also alter the antigen binding properties of the antibody. This alteration in antigen binding properties was directly attributed to the modification to the CH2 domain, as indicated by the following statement:

> [c]learly, the deletion of the CH2 domain has had an effect on the overall structure of the molecule, since both the formation of disulfide bonds in the adjacent H chains is affected along with binding properties of the V region (Gillies *et al.*, page 53, column 1, first sentence second paragraph).

From such teachings, one of skill in the art would have expected that modifications to the Fc region of a fusion protein as presently claimed, might adversely effect the activity of the CTLA4 portion of the fusion protein. For example, one of ordinary skill in the art might have expected, based on the Gillies *et al.* disclosure, that the claimed modifications to the Fc region might adversely effect the binding affinity of the CTLA4 region to B7. Applicants provide evidence in the instant application that the CTLA4-Ig fusion protein is not sensitive to such effects in the form of multiple examples of modified CTLA4-Ig fusion proteins which retain functionality. However, prior to this disclosure, the ability to make such modifications to CTLA4-Ig fusion proteins could not have been predicted with a reasonable expectation of success.

Claims 61, 62, 63, and 69

As a preliminary matter, claim 61 and the claims that depend therefrom require the presence of a CH1 domain. The cited references do not teach all the limitations of these claims as required by M.P.E.P. 2143. Specifically, the cited references fail to teach or suggest the claimed fusion protein molecules comprising an immunoglobulin constant region comprising the CH1 domain as required by claims 61, 62, 63, and 93. Thus, these claims are not obvious in view of the combination of these references.

Independent Claims 56 and 61 and Claims Depending Therefrom

While Linsley *et al.* generally describes the formation and expression of human CTLA4-immunoglobulin fusion protein, it does not teach or suggest substitutions in the Fc region at specific positions. In fact, Linsley *et al.* teaches that CTLA4 Ig is effective ***without*** any modifications to reduce complement activation and Fc receptor binding. Hence, the Linsley *et al.* reference is devoid of any teaching or suggestion that such a modification to a CTLA4 Ig fusion protein would be desirable, as required by the claims. The failure of the Linsley *et al.* reference to teach or suggest a critical element of the claimed ***modified*** CTLA4-immunoglobulin fusion protein, leads to a failure of the reference to teach or suggest the claimed compositions.

The remaining references fail to make up for this deficiency in the primary reference. The Office Action relies on the teachings of Freeman *et al.* and Gilles *et al.* for providing the motivation to combine the cited references. While Freeman *et al.* teaches modification of the CH2 domain in IgC to replace amino acids thought to be ***involved*** in binding to Fc receptor (see column 57), Freeman *et al.* does not disclose why such modifications would be desirable. Regarding Freeman *et al.*, the Office Action indicates that improvements in a CTLA4-Ig fusion protein which eliminate Fc mediated effector function would be obviously advantageous since the improvements were made in the B7-2 fusion proteins taught by Freeman. However, Freeman *et al.* does not teach the advantages of modification of the Fc region to enhance the *in vivo* use. Freeman *et al.* teaches CTLA4-Ig fusion proteins wherein the Ig portion of the fusion protein is unmodified. Freeman *et al.* also teaches B7-Ig fusion proteins in which the Ig portion of the fusion protein "may contain genetic modifications including those which reduce or eliminate effector activity inherent in the immunoglobulin structure" (Freeman *et al.*, column 54, lines 53-56). At no point does Freeman *et al.* indicate that such modifications are in any way more beneficial or preferred over equivalent fusion proteins which lack said modifications. At no point does Freeman *et al.* teach or suggest modification of CTLA4-Ig fusion protein to reduce or eliminate Fc mediated receptor activity. A CTLA4-Ig fusion protein was previously known in the prior art to function absent such modification. In the absence of indications as to the particular benefit in making additional modifications, one of ordinary skill in the art would not have been motivated to apply the modifications to the Fc region of the cited prior art to the CTLA4-Ig fusion protein.

The Office Action relies on the teachings of Gillies *et al.* as further providing motivation to one of skill in the art by disclosing the desirability of reducing Fc functions for *in vivo* applicability. However, the teachings of Gillies *et al.*, with respect to the *in vivo* applicability of Fc modifications do not readily apply to the present invention. The disclosure of Gillies *et al.* teaches modification of whole antibodies engineered for use in *in vivo* tumor imaging, more specifically engineered chimeric antibodies with human tumor specificity for use in *in vivo*

tumor imaging. The antibodies taught are engineered to reduce or eliminate Fc receptor binding and complement fixation. This modification is hypothesized to benefit *in vivo* tumor imaging, and similar applications. Gillies *et al.* states that "such (mutant) antibodies may be useful in applications, such as *in vivo* imaging of tumors, where the loss of effector function (*e.g.*, Fc receptor binding) is desired," and further teaches that such loss of effector function is beneficial for *in vivo* imaging of tumors because it reduces non-specific accumulation in the spleen. Gillies *et al.* does not teach that loss of Fc mediated effector functions is desirable in ***all in vivo*** applications. The actual teachings of Gillies *et al.* is of critical importance as to whether those teachings would motivate one of ordinary skill in the art to make the present invention, since the CTLA4-Ig fusion protein is used in applications which are significantly different from in *in vivo* imaging. In short, the specific advantage of reduced accumulation of the CTLA4-Ig fusion protein in the spleen, as indicated by the disclosure of Gillies *et al.*, is not of direct benefit in applications of the present invention. Absent teachings of a directly applicable benefit, one of ordinary skill in the art would not have been motivated by the disclosure of Gillies *et al.* to combine the teachings of the cited prior art to make the present invention.

Likewise, Canfield *et al.* teaches certain modifications to ***whole antibodies*** to examine the effect of amino acid substitutions on constant region mediated Fc receptor binding. The reference fails to teach or suggest that such modifications could be made in ***any*** Ig fusion protein, let alone a CTLA4 Ig fusion protein. Because all of the claims require the presence of a modified Cγ4 immunoglobulin constant region, it is Applicants' position that Canfield *et al.* actually teaches away from the claimed invention. Canfield *et al.* teaches that IgG4 has a lower affinity for the Fc receptors than either IgG1 or IgG3, hence there would be no motivation to modify such a constant region. In addition, the modification to IgG4 by Canfield *et al.* (substituting a Leu at position 234) actually increased binding to FcγR1.

Lund *et al.* also teaches certain modifications to ***whole antibodies*** to examine the effect of amino acid substitutions on constant region mediated Fc receptor binding. As with Canfield *et al.*, this reference fails to teach or suggest that such modifications could be made in ***any*** Ig

fusion protein, let alone a CTLA4 Ig fusion protein. Furthermore, Lund *et al.* teaches as the preferred modifications substitution at positions 234 and 237, rather than 235 and 237, as required by claims 92-94.

The disclosures of Canfield *et al.*, Lund *et al.*, and Duncan *et al.* do not teach any benefits associated with eliminating Fc mediated responses in an antibody or an antibody fusion protein. Canfield *et al.* and Lund *et al.* teach regions of whole antibodies which are involved in binding to Fcγ receptors. Duncan *et al.* teach specific residues in the CH2 domain of a whole antibody which are important in binding to C1q. None of these references teach any benefits associated with making modifications in an antibody to reduce Fc mediated responses, nor do they teach or suggest that such modifications could or should be made in an Ig fusion protein. Therefore, one of skill in the art would not have been motivated to combine the teachings of Freeman *et al.*, Gillies *et al.*, Canfield, *et al.*, Lund *et al.*, and Duncan *et al.* to produce the present invention.

The Office Action has failed to point to any suggestion in the art to alter the CTLA4 Ig fusion protein disclosed by Linsley *et al.*, nor any of the other references cited, as claimed by Applicants to reduce complement activation or Fc receptor binding as required by the claims. For all of the foregoing reasons, Applicants submit that the claimed invention is unobvious over the cited art.

SUMMARY

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

If a fee is due, please charge our Deposit Account No. 12-0080, under Order No. RPN-001CN from which the undersigned is authorized to draw.

Dated: March 3, 2004

Respectfully submitted,

By _____

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